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Maternal Multiple Micronutrient Supplementation Has Limited Impact on Micronutrient Status of Bangladeshi Infants Compared with Standard Iron and Folic Acid Supplementation[1–3]

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Abstract

Knowledge about the impact of maternal food and micronutrient supplementation on infant micronutrient status is limited. We examined the effect of maternal food and micronutrient supplementation on infant micronutrient status in the Maternal and Infant Nutrition Interventions in Matlab Trial. Pregnant women (n = 4436) were randomized to Early or Usual promotion of enrollment in a food supplementation program. In addition, they were randomly allocated to 1 of the following 3 types of daily micronutrient supplements provided from wk 14 of gestation to 3 mo postpartum: *1*) folic acid and 30 mg iron (Fe30Fol); *2*) folic acid and 60 mg iron; or *3*) a multiple micronutrient including folic acid and 30 mg iron (MMS). At 6 mo, infant blood samples (n = 1066) were collected and analyzed for hemoglobin and plasma ferritin, zinc, retinol, vitamin B-12, and folate. The vitamin B-12 concentration differed between the micronutrient supplementation groups (P = 0.049). The prevalence of vitamin B-12 deficiency was lower in the MMS group (26.1%) than in the Fe30Fol group (36.5%) (P = 0.003). The prevalence of zinc deficiency was lower in the Usual food supplementation group (54.1%) than in the Early group (60.2%) (P = 0.046). There were no other differential effects according to food or micronutrient supplementation groups. We conclude that maternal multiple micronutrient supplementation may have a beneficial effect on vitamin B-12 status in infancy. J. Nutr. 140: 618–624, 2010.

Introduction

Micronutrient deficiency during infancy is a serious public health problem in low-income countries. Iron, zinc, vitamin A, and vitamin B-12 are some of the nutrients of concern. Iron deficiency anemia in infancy has long-lasting negative effects on neurodevelopment and behavior (1). Zinc deficiency is a considerable contributor to mortality, burden of disease (2), and growth impairment (3). Similarly, vitamin A is crucial for normal development of immune function and high-dose vitamin A supplements have a major impact on infectious diseases mortality (4,5). Deficiency of vitamin B-12 has negative consequences for neurodevelopment (6).

Infant iron, zinc, vitamin A, and vitamin B-12 status is associated with maternal status of these nutrients. Neonatal serum ferritin has been related to maternal hemoglobin (Hb)[9] in most studies (7) and there is some evidence of improved iron status in response to maternal iron supplementation (8). Zinc

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[9] Abbreviations used: CRP, C-reactive protein; Early, promoted to enroll into a food supplementation program as soon as pregnancy was diagnosed; EBF, exclusive breast-feeding; Fe30Fol, 30 mg iron and folic acid supplement; Fe60Fol, 60 mg iron and folic acid supplement; Hb, hemoglobin; ICDDR,B, International Centre for Diarrhoeal Disease Research, Bangladesh; IQR, interquartile range; MINIMat, Maternal and Infant Nutrition Interventions in Matlab; MMS, multiple micronutrient supplement; Usual, not promoted to enroll into a food supplementation program early in pregnancy: VAD, marginal vitamin A status.

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supplementation during pregnancy increases neonatal cord blood zinc (9). Maternal vitamin A status is related to concentrations of vitamin A in breast milk (10) and maternal vitamin A supplementation increases infant vitamin A status (11,12). Maternal vitamin B-12 status is highly associated with vitamin B-12 status of newborns (13) and with breast milk concentrations of vitamin B-12 (14), whereas the folate content of breast milk is relatively independent of maternal folate status (15). Thus, in settings where micronutrient deficiencies are common in women of childbearing age, infants are at high risk of developing iron, zinc, vitamin A, and vitamin B-12 deficiency and could benefit from maternal supplementation of these nutrients.

Because micronutrient deficiencies often coexist (16,17), a multiple micronutrient supplement (MMS) for use during pregnancy and lactation has been developed by UNICEF and other agencies (18). This particular supplement, containing 30 mg iron, 400 μg folic acid, and ~1 Recommended Daily Allowance of 13 other micronutrients, has been evaluated in relation to pregnancy outcomes in the trial Maternal and Infant Nutrition Interventions in Matlab (MINIMat; ISRCTN16581394) in Bangladesh and in other countries (19). In the MINIMat trial, there was no differential effect of multiple micronutrients on birth weight compared with iron folic acid supplements (personal communication, L-Å. Persson, Department of Women's and Children's Health, Uppsala University), whereas other studies reported a beneficial effect of MMS (20–22). From other studies where similar formulations of MMS were compared with iron folic acid supplements, improvements in maternal status of some micronutrients are reported (23), but not of iron (24,25). We are not aware of any studies of the effect of this particular maternal MMS on infant micronutrient status. An immediate question is whether a shift from the prenatal supplement currently recommended by WHO (60 mg iron and 400 μg folic acid) (26) to one with multiple micronutrients would improve micronutrient status of infants. Of special concern is iron, as this multiple micronutrient formulation contains only one-half of the amount of iron recommended by WHO. Although there is evidence for interactions between micronutrients in supplements, interactions between food and micronutrient supplements are not well explored. Besides providing micronutrients, food and food supplements can enhance or inhibit absorption and utilization of micronutrients from a micronutrient supplement. In this paper, we assessed whether infant micronutrient status was affected by 3 types of maternal micronutrient supplement and time of enrollment in a food supplementation program. The food supplement was provided during pregnancy and the micronutrient supplementation ended at 3 mo postpartum. We assessed infant micronutrient status at 6 mo, because this is the time when deficiencies of some nutrients starts to develop.

Methods

Setting. This study is a follow-up of the MINIMat trial in Matlab, rural Bangladesh, where a health and demographic surveillance system is operating. In the study area, community health research workers from the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B) visit households monthly to collect data. In this region, most people depend on agriculture, fishing, or day labor. The staple food rice is eaten with pulses, vegetables, or, when affordable, fish. Breast-feeding is almost universal during infancy. According to the national policy, children 9–59 mo should be supplemented with vitamin A. It has been reported that 13% of mothers in rural Bangladesh receive a vitamin A dose within 2 mo postpartum (27). The government of Bangladesh aims to provide all underweight (BMI <18.5 kg/m^2) pregnant women with a food supplement through the National Nutrition Program. They also recommend that all pregnant women, regardless of nutritional status, take a daily supplement of 60 mg iron and 400 μg folic acid.

Participants and protocol. The study was a community-based trial with individual randomization to be promoted to early enrollment into a food supplementation program (Early) or to receive no such promotion (Usual). In addition, women were allocated to 1 of 3 types of micronutrient supplements in a 2 by 3 design. A computerized tracking system assigned women to 1 of the 6 groups in blocks of 12. The 3 types of daily micronutrient supplement capsules were: *1*) 30 mg iron (fumarate) + 400 μg folic acid (Fe30Fol); *2*) 60 mg iron (fumarate) + 400 μg folic acid (Fe60Fol); and *3*) 30 mg iron (fumarate), 400 μg folic acid, 800 μg RE vitamin A (retinyl acetate), 5 μg vitamin D (cholecalciferol), 10 mg vitamin E (α-tocopherol acetate), 70 mg vitamin C, 1.4 mg thiamine (mononitrate), 1.4 mg riboflavin, 18 mg niacin, 1.9 mg vitamin B-6 (pyridoxine hydrochloride), 2.6 μg vitamin B-12 (cyanocobalamin), 15 mg zinc (sulfate), 2 mg copper (sulfate), 65 μg selenium (sodium selenite), and 150 μg iodine (potassium iodide) (MMS). To ensure blinding, the capsules were identical in appearance. Enrolled women visited a subcenter clinic at ~14 wk of gestation and had a blood sample taken and analyzed for Hb concentration by HemoCue. Women with Hb <80 g/L were excluded from the study and referred to Matlab hospital. All other women were supplied with micronutrient capsules monthly by field research assistants up to 3 mo after delivery.

The food supplement was available at community nutrition centers 6 d/wk as part of the National Nutrition Program. The supplement consisted of roasted rice powder, roasted pulse powder, molasses, and soybean oil and had a total energy content of 2500 kJ. The study team encouraged women who had been randomized to the Early group to start attending the food supplementation program immediately after confirmation of pregnancy. The community nutrition centers were notified about which women were pregnant and should be enrolled in the program. Women who were randomized to the Usual group did not receive this promotion and started taking the food supplement at the time of their own choosing.

We recruited and randomized 4436 pregnant women to the food and micronutrient supplementation interventions between November 2001 and October 2003. ICDDR,B staff informed eligible women about the study and those who wanted to participate gave their written consent. The ethical review committee of ICDDR,B, Dhaka, Bangladesh approved the study.

Data collection. Trained interviewers used structured, pilot-tested questionnaires to collect socioeconomic and demographic information at baseline. As a measure of socioeconomic status, the MINIMat team constructed a wealth index based on household assets (28). We used data from an electronic drug event monitoring system (eDEM) to measure compliance to micronutrient supplementation. All bottles with capsules were equipped with a microprocessor that recorded every opening of the cap. Food supplement intake was assessed by monthly recall up to wk 34 of pregnancy.

Interviewers visited all women monthly during pregnancy. Infants weighing >1000 g within 72 h of birth were followed-up every month to 6 mo after delivery. At 6 mo postpartum, all women were encouraged to go to an ICDDR,B-operated health center where paramedics took venous blood samples from the infants. Plasma samples from 1066 infants were stored in Matlab at −70°C and shipped to the laboratory at University of California, Davis for analyses. Hb was measured by HemoCue at the time of sampling. Because the equipment for Hb testing was not in place, assessment of Hb was delayed until December 2003 (first blood sample taken in May 2003), resulting in 796 analyzed samples.

Assessment of micronutrient status. Plasma ferritin was analyzed by RIA (Diagnostic Products) and the detection level was set to 0.1 μg/L. Elevated plasma C-reactive protein (CRP) (>10 mg/L) was detected by radial immunodiffusion (The Binding Site). Plasma zinc concentration

was analyzed by flame atomic absorption spectroscopy (29). Plasma retinol was determined by isocratic reverse-phase HPLC and UV detection (30). Plasma vitamin B-12 and folate were quantified simultaneously with a SimulTRAC-SNB RIA kit (MP Biomedicals) through the principle of competitive protein binding. We defined anemia as Hb <105 g/L and iron deficiency as plasma ferritin <9 μg/L (31), but analyses were also performed with Hb <110 g/L and plasma ferritin <12 μg/L (26). Plasma zinc <9.9 μmol/L was categorized as zinc deficiency (32) and a plasma retinol concentration <0.70 μmol/L was regarded as indicative of marginal vitamin A status (VAD) (33). To define vitamin B-12 deficiency and folate deficiency, we applied the commonly used cutoff values of plasma vitamin B-12 <150 pmol/L and plasma folate <6.8 nmol/L.

Statistical methods. We used Pearson chi-square tests, ANOVA, and Kruskall-Wallis tests to evaluate differences in maternal and infant characteristics by supplementation groups. Potential interactions between food and micronutrient supplementation were evaluated by including an interaction term in general linear models. To examine the differential effects of Fe30Fol, Fe60Fol, and MMS on infant micronutrient status, we used 1-way ANOVA with Bonferroni post hoc tests. Because plasma ferritin and vitamin B-12 were not normally distributed, differences between supplementation groups were evaluated after log transformation of these variables. Plasma ferritin values <1 μg/L were set to 1 μg/L before log transformation. We used ANCOVA to adjust for elevated CRP. Differences in prevalence of deficiency were assessed with Pearson chi-square tests. To assess the risk for deficiency, we conducted preplanned logistic regressions, adjusting for elevated CRP where appropriate. We stratified analyses for infant gender. Data in the text are means ± SD or median [interquartile range (IQR)]. Statistical tests were evaluated with a probability test of $P < 0.1$ for interactions and $P < 0.05$ for main effects. We used SPSS (version 14.0) for all statistical analyses.

Results

Of the 4436 women enrolled in the study, 3267 gave birth to a singleton infant whose weight was measured at birth (**Supplemental Fig. 1**). This led to the invitation of the infant to participate in the follow-up and 2756 infants were followed to 6 mo. At that time, many of the women did not accept the invitation to come to the clinic for blood sampling of their infants and others refused blood sampling at the clinic, resulting in blood collected from 1066 infants (study sample). The numbers of the different micronutrient analyses are lower due to laboratory losses and exclusion of implausible values (0–2/micronutrient). In the study sample, baseline characteristics of women and infants were similar across micronutrient supplementation groups (**Table 1**).

In the study sample, exclusive breast-feeding (EBF) for ≥4 mo was more common (52%) than for those infants not included in the sample (36%) ($P < 0.001$). Other characteristics did not differ between infants with or without a blood sample. Women in our sample had lower education ($P = 0.030$), lower parity ($P = 0.001$), and were slightly older at enrollment (26.3 ± 6.2) than women whose infants were not included in the study sample (25.6 ± 5.9) ($P = 0.001$). Women in the sample had lower Hb at wk 14 (116 ± 12 g/L) than those not in the sample (117 ± 13 g/L) ($P = 0.022$). Other maternal characteristics such as BMI and socioeconomic status did not differ between our sample and other women enrolled in MINIMat.

Intakes of food supplement and micronutrient capsules did not differ between micronutrient supplementation groups (Table 1). Participants in the Early food supplementation group started attending the community nutrition centers in the first trimester and women in the Usual group commonly started in the second trimester. Women in the Early group reported having consumed more food packages from wk 8 to wk 34 [105 (67–129)] than participants in the Usual group [66 (32–87)] ($P < 0.001$).

Among infants in this population, zinc deficiency [57% (601/1050)] was the most common micronutrient deficiency followed by anemia [47% (374/796)] and vitamin B-12 deficiency [31% (323/1033)]. Deficiencies of vitamin A [23% (237/1026)] and iron [21% (217/1058)] were also prevalent, whereas folate deficiency was uncommon [1% (10/1031)]. We found no interaction between type of micronutrient supplement and food supplement group on the concentration of any micronutrient. Thus, the effects of food and micronutrient interventions were evaluated independently.

The plasma vitamin B-12 concentration differed among the supplementation groups ($P = 0.049$) and tended to be higher in the MMS group than in the Fe60Fol group ($P = 0.054$) (**Table 2**). Similarly, there were differences in prevalence of vitamin B-12 deficiency between micronutrient groups, with a lower prevalence in the MMS group than in the Fe30Fol group ($P = 0.003$) (**Table 3**). However, the risk of vitamin B-12 deficiency did not differ between the micronutrient supplementation groups (**Table 4**). Analyses stratified by gender indicated that differences between the micronutrient supplementation groups in prevalence of vitamin B-12 deficiency were due to an effect in girls only. Among girls, 43% (73/171) were deficient in the Fe30Fol group and 30% (52/175) in the MMS group ($P = 0.031$), whereas there was no differential effect of type of supplements on prevalence of vitamin B-12 deficiency in boys. Although not significant, adjusted analyses indicated that MMS tended to be associated with higher plasma ferritin ($P = 0.06$; Table 2) and reduced risk of VAD ($P = 0.09$; Table 4).

Concentrations of Hb and ferritin, zinc, retinol, or folate in plasma did not differ across micronutrient supplementation groups in the total sample (Table 2) or when the data were stratified by infant gender. The prevalence (Table 3) or risk (Table 4) of anemia and deficiency of iron, zinc, vitamin A, and folate in infancy did not differ among micronutrient supplementation groups in the total sample or in either gender strata. These results remained the same when we used the WHO cutoffs of Hb <110 g/L and plasma ferritin <12 μg/L (26) to define anemia and iron deficiency.

The prevalence of zinc deficiency in the Early food supplementation group [60% (324/538)] was higher than in the Usual group [54% (277/512)] ($P = 0.046$). This finding was supported by an increased risk of zinc deficiency in the Early group, although it did not reach significance [OR = 1.28 (95% CI: 1.0, 1.6) ($P = 0.051$). Randomization to Early introduction of food supplementation did not result in any other differential effects on infant micronutrient status.

Discussion

Anemia and deficiencies of zinc and vitamin B-12 were high in infants in this population. In our analyses of maternal micronutrient supplementation and infant micronutrient status, MMS was associated with improved vitamin B-12 status. Zinc deficiency was more common in infants whose mothers were randomized to the Early food supplementation group than in infants whose mothers were in the Usual food group. No other differential effects between maternal food or micronutrient supplementation groups were found. For infant micronutrient status, maternal supplementation with 30 mg iron and folic acid did not differ from supplementation with 60 mg iron and folic acid.

TABLE 1 Maternal and infant characteristics by type of maternal micronutrient supplement[1]

	Fe30Fol	Fe60Fol	MMS
n	361	342	363
Randomized to Early food supplementation, *n (%)*	191 (52.9)	161 (47.1)	194 (53.4)
Maternal age, *y*	26.4 ± 6.0	26.3 ± 6.1	26.3 ± 6.4
Maternal BMI,[2] *kg/m²*	20.2 ± 2.6	20.1 ± 2.7	20.2 ± 2.6
Maternal Hb,[3] *g/L*	116 ± 12	115 ± 13	116 ± 13
Maternal education, *n (%)*			
No education	110 (30.5)	107 (31.3)	121 (33.3)
≤5 y	87 (24.1)	85 (24.9)	85 (23.4)
≥6 y	164 (45.4)	150 (43.9)	157 (43.3)
Parity,[4] *n (%)*			
0	104 (28.8)	108 (31.7)	123 (31.5)
1–2	193 (53.5)	163 (47.8)	168 (46.3)
≥3	64 (17.7)	70 (20.5)	72 (19.3)
Micronutrient supplement intake[5]	88 (58–105)	87 (56–107)	76 (52–103)
Food supplement intake[6]	84 (55–112)	79 (44–106)	85 (46–114)
Male infant, *n (%)*	184 (51.0)	184 (53.8)	183 (50.4)
Birth weight, *g*	2693 ± 424	2690 ± 400	2708 ± 385
Low birth weight (<2500 g), *n (%)*	120 (33.2)	97 (28.4)	99 (27.3)
EBF duration ≥4 mo, *n (%)*	185 (51.2)	181 (52.9)	185 (51.0)
Elevated CRP at 6 mo, *n (%)*	44 (12.3)	46 (13.5)	56 (15.6)

[1] Values are *n* (%), mean ± SD, or median (IQR). Variables did not differ between supplementation groups
[2] *n* = 360 (Fe30Fol).
[3] *n* = 333 (Fe30Fol), 316 (Fe60 Fol), and 339 (MMS).
[4] *n* = 341 (Fe60Fol).
[5] eDEM count, wk 14–30, *n* = 317 (Fe30Fol), 308 (Fe60 Fol), and 324 (MMS).
[6] Recall of food packages consumed wk 14–34, *n* = 360 (Fe30Fol), 341 (Fe60 Fol), and 362 (MMS).

Maternal supplementation with MMS could have influenced infant vitamin B-12 status both through increased infant prenatal stores of the vitamin (13) and through increased concentrations of vitamin B-12 in breast milk (14). Because the supplements were given during pregnancy and continued to 3 mo postpartum, we cannot distinguish between pre- and postnatal effects. In our study, the effect on infant vitamin B-12 status appeared not to be due to improved maternal B-12 status, as there were no differential effects of the supplements on maternal vitamin B-12 status (34). The impact of maternal vitamin B-12 supplementation on infant vitamin B-12 status has been reported previously (35). The dose of vitamin B-12 in the MMS group might have been too low to reduce the risk for vitamin B-12 deficiency or a potential effect was gone at the time of assessment of infant vitamin B-12 status. The effect size of our finding is small and does not indicate that infant vitamin B-12 status would be improved by administering MMS instead of the currently recommended Fe60Fol, because the MMS and Fe60Fol groups did not differ. We are not aware of any biological mechanism for our finding that the lower prevalence of vitamin B-12 deficiency in the MMS group was present in the strata in girls only. However, sex differences in response to supplementation of several nutrients has been described previously (36).

We did not observe a differential effect of type of micronutrient supplementation on infant vitamin A status, although previous studies indicate an effect of maternal vitamin A supplementation on infant vitamin A status (10,12). Although not significant, the risk of VAD was about 30% lower in the MMS group than in the iron and folic acid groups ($P = 0.09$; Table 4). One likely explanation for the lack of effect is that the dose of vitamin A in the MMS may have been too low to affect

TABLE 2 Blood Hb and plasma micronutrient concentrations of infants by type of maternal micronutrient supplement[1]

	Fe30Fol		Fe60Fol		MMS		*P*-value
	n		*n*		*n*		
Hb, *g/L*	202	106 (98–113)	202	105 (97–112)	212	105 (98–113)	0.55[3]
Ferritin,[2] *μg/L*	356	21.3 (10.4–40.5)	340	21.5 (9.7–41.9)	360	24.1 (12.3–47.4)	0.06[3]
Zinc, *μmol/L*	355	9.6 (8.1–10.8)	337	9.6 (8.2–11.0)	357	9.5 (8.2–10.8)	0.49[3]
Retinol, *μmol/L*	341	0.88 (0.70–1.06)	331	0.84 (0.70–1.04)	349	0.89 (0.74–1.09)	0.18[3]
Vitamin B-12,[2] *pmol/L*	351	190 (123–305)	329	197 (126–322)	353	221 (145–324)	0.05[4]
Folate, *nmol/L*	353	30.0 (22.1–37.9)	328	30.4 (22.4–38.8)	350	31.2 (22.1–31.1)	0.64[4]

[1] Values are median (IQR).
[2] Analyses with log-transformed variables.
[3] Adjusted for elevated CRP with ANCOVA.
[4] Unadjusted analyses with 1-way ANOVA.

TABLE 3 Prevalence of micronutrient deficiencies in infants at 6 mo by type of maternal micronutrient supplement

	Fe30Fol	Fe60Fol	MMS	*P*-value[1]
	n (%)			
Anemia[2]	114 (44.0)	131 (49.6)	129 (47.3)	0.43
Iron deficiency[3]	80 (22.4)	75 (22.0)	62 (17.2)	0.16
Zinc deficiency	207 (58.1)	189 (56.1)	205 (57.4)	0.86
VAD	86 (25.0)	81 (24.5)	70 (19.9)	0.22
Vitamin B-12 deficiency	128 (36.5)	103 (31.3)	92 (26.1)	0.01
Folate deficiency	5 (1.4)	4 (1.2)	1 (0.3)	0.27

[1] Differences assessed with chi-square tests.
[2] Hb <105 g/L, n = 796.
[3] Plasma ferritin <9 μg/L.

infant vitamin A status. It is also possible that an increase in breast milk retinol concentrations in women supplemented with MMS led to improvements in infant vitamin A status early in infancy but did not last to be detected at 6 mo.

Despite the difference in iron content between the micronutrient supplements Fe60Fol and Fe30Fol and MMS, we did not find a significant differential effect in infant iron status between micronutrient supplements. A small effect was indicated favoring MMS. An effect of MMS is plausible due to improved maternal iron uptake and utilization of iron from MMS due to its content of vitamin C and A. Maternal iron supplementation is likely to affect infant iron status through increased iron stores at birth, because iron concentration of breast milk is not affected by maternal iron status. Infant iron status did not differ between infants whose mothers received 30 mg iron and those whose mothers received the currently recommended dose of 60 mg iron. This suggests that even in a setting where iron-fortified foods for infants are uncommon, supplementing mothers with a lower dose of iron would not cause poorer infant iron status. Anemia rates are much higher than iron deficiency rates in this population and causes of infant anemia other than iron deficiency should be investigated.

The lack of impact of prenatal zinc supplementation compared with iron and folic acid supplementation on infant zinc status has been noted before (37). One explanation may be the insensitivity of plasma zinc as an indicator of zinc deficiency. However, the fluctuation in plasma zinc is likely to cause a random error, similar across supplementation groups. The amount of zinc in the maternal supplement may not have been sufficient to prevent zinc deficiency of women in our study, because zinc status did not differ between micronutrient supplementation groups (34). At 6 mo, feeding practices, morbidity, and growth are important determinants of infant micronutrient status, including zinc. Almost all infants in our study were breast-fed and a longer duration of EBF was associated with higher zinc concentrations (38). Birth weight did not differ between the micronutrient supplementation groups in our study. However, because higher weight and larger body size in infants born to women supplemented with MMS compared with iron folic acid has been observed elsewhere (39), it is possible that the MMS supplement increased postnatal growth at the expense of infant zinc status.

TABLE 4 Risk for deficiency in infants at 6 mo by type of maternal micronutrient supplement[1]

	Fe60Fol[2] vs. Fe30Fol		Fe60Fol[2] vs. MMS	
	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
Anemia[3]	0.86 (0.58, 1.3)[4]	0.48[4]	0.98 (0.67, 1.4)[4]	0.94[4]
Iron deficiency[5]	1.0 (0.72, 1.5)[4]	0.87[4]	0.76 (0.52, 1.1)[4]	0.15[4]
Zinc deficiency	1.1 (0.81, 1.5)[4]	0.53[4]	1.0 (0.77, 1.4)[4]	0.77[4]
VAD	1.1 (0.73, 1.5)[4]	0.79[4]	0.72 (0.49, 1.0)[4]	0.09[4]
Vitamin B-12 deficiency	1.3 (0.92, 1.7)	0.16	0.77 (0.55, 1.1)	0.13
Folate deficiency	1.2 (0.31, 4.4)	0.82	0.23 (0.03, 2.1)	0.19

[1] Binary logistic regression, unadjusted unless indicated.
[2] Reference group.
[3] Hb <105 g/L.
[4] Adjusted for elevated CRP.
[5] Plasma ferritin <9 μg/L.

It was unexpected that randomization to promotion of early enrollment into a food supplementation program was associated with a higher prevalence of zinc deficiency than no promotion of early enrollment. This should not be interpreted as a caution for providing access to food supplementation early in pregnancy. The effect of maternal food supplementation on infant nutritional status should be evaluated using several indicators and zinc status alone may not be a suitable indicator. It is possible that early enrollment in the food supplementation program increased infant growth and thereby the requirements for zinc. Another potential mechanism is that the food supplement that was low in micronutrients (40) replaced other foods, resulting in lower total maternal zinc intake. Because the effect size was small, our finding probably has limited implications for food supplementation programs.

The strengths of this study were the randomized design and large sample size that rendered power enough to detect even small differential effects of the different types of supplements on infant micronutrient status. We calculated the differences in infant micronutrient status that we could detect with the smallest sample size in any supplementation group (n = 202). Using this sample size, 80% power, and 95% probability, we were able to detect a difference of 0.3 SD between supplementation groups. We judge that a smaller difference than 0.3 SD is of limited public health importance and we do not consider limited sample size as a plausible reason for nonsignificant results. In the presence of infection and inflammation in adults, plasma ferritin is elevated as part of the acute phase response, whereas concentrations of plasma zinc and plasma retinol are reduced. We used elevated CRP to adjust for changes in biomarkers as a result of infections. The MINIMat study had high follow-up rates, but many women refused blood sampling of the child. The small differences in characteristics between women who allowed blood sampling of the child and those who refused may have a limited impact on external validity. The results represent what can be expected in this population with its specific characteristics.

Despite prenatal food supplementation and maternal micronutrient supplementation to 3 mo postpartum, infant micronutrient deficiencies at 6 mo were prevalent in this population. Use of maternal MMS may lead to a small reduction in vitamin B-12 deficiency. This effect may be important, because vitamin B-12 deficiency is prevalent in this population. For the other 2 most common micronutrient-related problems, zinc deficiency and anemia, there was no differential effect of the micronutrient supplements. We conclude that maternal MMS during pregnancy and to 3 mo postpartum does not solve the problem of the high prevalence of micronutrient deficiency in 6-mo-old infants. Other measures to improve infant Hb and status of zinc, vitamin B-12, iron, and vitamin A in this population are needed. Although we did not see a large differential impact of the supplements, the different nutritional interventions in the

MINIMat trial have been related to outcomes such as reduced infant mortality (personal communication, L-Å. Persson, Department of Women's and Children's Health, Uppsala University), slightly improved cognitive development (41), and associations with maternal-infant feeding interactions (42). Whether infant micronutrient status leads to these functional outcomes requires further analyses.

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